

The Effect of Gonadotropin Releasing Hormone Administration on Fertility and Embryonic Loss in Goats during the Anoestrus Period

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ABSTRACT

Background: Following the induction of oestrus out of season in small ruminants, low fertility and variations in fertility rates are associated with embryonic losses. One of the main causes of embryonic loss is luteal dysfunction. Gonadotropin Releasing Hormone (GnRH) supports the luteal structure, and increasing progesterone levels may be beneficial in terms of promoting embryonic life. The main objective of the present study was to evaluate the efficacy of GnRH administration following an oestrus induction protocol in the anoestrus season for preventing embryonic loss in goats having failure to conceive during the season.

Materials, Methods & Results: In the study, 106 Damascus goats aged 3-5 years and weighing 45-60 kg were used. The oestrus of 106 goats in the anoestrous group was stimulated with progesterone and pregnant mare serum gonadotropin (PMSG) treatment. Out of breeding season, goats were divided into the 4 following groups: GnRH₀ (n = 27), GnRH₇ (n = 26), GnRH₀₊₇ (n = 27) and control (n = 26). In each goat, an intravaginal sponge (IS) containing 20 mg of fluorogestone acetate (FGA) was placed into the vagina and left for 9 days. With the withdrawal of the sponge, 550IU PMSG and 125 µg of d-cloprostenol were injected intramuscularly. Oestrus detection was made via teaser bucks for 3 days starting 24 h after withdrawal of the IS. Eighteen bucks known to be fertile were used for breeding. Goats in the oestrus period were mated via natural breeding. The GnRH analogue lecorelin was injected intramuscularly at breeding in the GnRH₀ group, on day 7 post-breeding in the GnRH₇ group, and both at breeding and on day 7 post-breeding in the GnRH₀₊₇ group. No injections were given to the control group. Blood samples for progesterone measurement were taken by jugular vena puncturing on days 3, 6, 7, 10, 13, 16, and 19 after breeding from 10 randomly chosen goats in all groups. The goats with a level of > 3.5 ng/mL of progesterone on day 21 post-breeding were evaluated as pregnant. Pregnancy was also viewed on day 50 after breeding by real-time ultrasonography (USG) with a 5-7.5 MHz convex probe. The oestrus rate was 96.23% (102/106) in the goats. The rates of onset of oestrus between 36-48 h, 48-60 h and 60 h and beyond were 38.7% (41/106), 21.7% (23/106) and 35.8% (38/106), respectively. The total pregnancy rate was 35.8% (38/106). There were no statistically significant differences ($P > 0.05$) found for the pregnancy rate, embryonic death rate or progesterone concentration of the groups. However, serum progesterone levels were statistically different in the GnRH₇ group compared with the control group ($P < 0.05$).

Discussion: After synchronisation, various anti-luteolytic strategies can be used to support corpus luteum development and elevate progesterone concentration in the luteal phase to decrease embryonic loss and increase reproductive performance. Therefore, application of GnRH to support the luteal structure and to increase progesterone levels may be beneficial in terms of supporting embryonic life. The results showed that GnRH treatment on the day 7 post-breeding following oestrus induction, including FGA and PMSG, can increase serum progesterone levels in Damascus goats in the anoestrus period. However, following oestrus induction in the anoestrus period, it was seen that GnRH treatment at breeding or on day 7 after breeding did not have any positive effect on embryonic loss or reproductive performance. In conclusion, it was considered that this protocol could be implemented successfully, yielding a 35% pregnancy rate in Damascus goats in the anoestrus period, but embryonic loss must be deeply studied in detail.

Keywords: anoestrus, embryonic loss, oestrous induction, GnRH, goat.

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INTRODUCTION

From goats that fail to conceive during the breeding season, pregnancy can be achieved with applications performed outside the breeding season [9,15]; for instance, gonadotropin combined with progestogens is widely used for the induction of oestrus [20,32]. It has been reported that the fertility rate in the anoestrus period varies between 33% and 77% after these applications [10]. Following the induction of oestrus outside the breeding season, low fertility and variations in the fertility rate between studies are caused by embryonic loss [10,14]. The main reasons for embryonic loss are luteal function deficiency, embryo-uterine asynchronisation and insufficient embryo development [16].

Different therapeutic strategies have been implemented to reduce embryonic loss and increase fertility performance after synchronisation protocols. Binelli *et al.* [6] classified anti-luteolytic strategies into 6 categories, one of which is to increase the luteal progesterone level. Here, luteotrophic hormones, such as GnRH or human chorionic gonadotropin (hCG), are used in the early luteal phase or the late luteal phase. Administering these hormones in the luteal phase provides accessory corpus luteum formation by acting directly or indirectly in the ovaries, resulting in an increased serum progesterone concentration [8,16]. In many ruminants, the administration of GnRH during mating or prior to maternal recognition is considered to support embryo viability by improving luteal function, inducing the luteinisation of developing follicles, and increasing the blood progesterone level [21,24]. This study aimed to determine the effect of GnRH administration following an oestrus induction protocol on fertility and embryonic loss in goats.

MATERIALS AND METHODS

Animals

This study was carried out on a commercial goat farm (Pan Hayvancılık, Nurdağı/Gaziantep) located at a latitude of 36°90'N and a longitude of 37°15'E. In this study, 106 Damascus goats aged 3-5 years and weighing 45-60 kg were used. All goats had been mated in the preceding breeding season, but they did not become pregnant. The daily milk average of the goats was 1.75 L. The goats were fed by ration with 2,800 kcal/kg ME and 18% crude protein. Water was given *ad libitum*.

Oestrus induction and study design

Out of breeding season, oestrus was stimulated with an intravaginal sponge (IS) containing 20 mg of fluorogestone acetate¹, PMSG² and d-cloprostenol³. The IS was placed into the vagina and left for 9 days. With the withdrawal of the IS, 550IU PMSG² and 125 µg of d-cloprostenol³ were injected intramuscularly. Oestrus detection was made by teaser bucks for 3 days, starting 24 h of withdrawal of the IS. Eighteen bucks known to be fertile were used for breeding. Goats in oestrus were mated by natural breeding within 1 h. The goats were divided into the 4 following groups: GnRH₀ (n = 27), GnRH₇ (n = 26), GnRH₀₊₇ (n = 27) and control (n = 26). GnRH analogue 25 µg lecorelin³ was injected intramuscularly at breeding in the GnRH₀ group, on day 7 post-breeding in GnRH₇ group, and both at breeding and on day 7 post-breeding in GnRH₀₊₇. No injection was given in the control group.

Serum samples and progesterone measurements

Blood samples for progesterone measurement were taken into anticoagulated tubes⁴ by jugular vena puncturing on days 3, 6, 7, 10, 13, 16 and 19 days after breeding from 10 randomly chosen goats in all groups. Blood sampling was also performed for all goats on day 21. Blood samples were centrifuged⁵ for 10 min at 2000 g. Separated serum samples were kept in a freezer at -20 °C until analysis. Serum progesterone levels were analysed using an electrochemiluminescent method in an autoanalyzer⁶.

Pregnancy diagnosis

Pregnancy diagnosis was carried out both by means of progesterone at 21 days post-breeding and USG 50 days after breeding was performed, as previously described [13]. Goats with a level of > 3.5 ng/mL progesterone on day 21 post-breeding were evaluated as pregnant. In addition, pregnancy was viewed on day 50 after breeding using real-time USG⁷ with a 5-7.5 MHz convex probe.

Formulae for reproductive parameters

Oestrus rate: (Goats in oestrus/total number of goats) x 100

Onset of oestrus: Interval between withdrawal of sponge and breeding

Pregnancy rate: (Number of pregnant goats/total number of goats) x100

Parturition rate: (Number of goats delivering/ total Number of goats) x 100

Multiple birth rate: (Number of goats giving multiple birth/number of goats delivering) x 100

Litter size: (Number of kids born/number of goats delivering) x 100

Embryonic loss rate: (Number of pregnant goats determined by 21-day progesterone level) - (number of pregnant goats determined by positive USG positive at day 50)/number of pregnant goats in group x 100

Statistical analysis

The pregnancy rate, parturition rate, multiple pregnancy rate and litter size were compared with

the Chi-square test using SPSS version 22 software⁸. Progesterone values of different sampling days were also compared using a one-way analysis of variance (ANOVA) test (SPSS22).

RESULTS

The oestrus rate was 96.23% (102/106) in the goats. The rates of onset of oestrus at 36-48 h, 48-60 h and 60 h and beyond were 38.7% (41/106), 21.7% (23/106) and 35.8% (38/106), respectively. The total pregnancy rate was 35.8% (38/106). Fertility parameters of the GnRH₀, GnRH₇, GnRH₀₊₇ and control groups are given in Table 1. Progesterone values of goats on different days of pregnancy are presented in Table 2.

Table 1. Fertility parameters of GnRH₀, GnRH₇, GnRH₀₊₇ and Control groups.

Parameter	Groups			
	GnRH ₀	GnRH ₇	GnRH ₀₊₇	Control
Pregnancy rate* (%)	70.4 (19/27)	88.5 (23/26)	70.4 (19/27)	88.5 (23/26)
Pregnancy rate** (%)	33.3 (9/27)	38.5 (10/26)	25.9 (7/27)	46.2 (12/26)
Embryonic loss rate (%)	52.6 (10/19)	56.5 (13/23)	63.1 (12/19)	47.8 (11/23)
Parturition rate (%)	77.8 (7/9)	100 (10/10)	100 (7/7)	83.3 (10/12)
Multiple birth rate (%)	44.4 (4/9)	30 (3/10)	28.6 (2/7)	50 (6/12)
Litter size (x/n)	1.57 (11/7)	1.5 (15/10)	1.28 (9/7)	1.9 (19/10)

*According to 21st day progesterone level; **According to USG examination on 50th day. Differences between groups were not statistically important. ($P > 0.05$).

Table 2. Progesterone values of goats on different days of pregnancy (ng/mL).

Day of pregnancy	Groups			
	GnRH ₀	GnRH ₇	GnRH ₀₊₇	Control
3	6.65 ± 1.40	4.56 ± 0.74 ^c	5.59 ± 0.95	7.93 ± 1.97 ^b
6	6.15 ± 1.21	6.50 ± 1.31 ^{bc}	9.57 ± 0.61	5.97 ± 1.33 ^b
7	6.55 ± 1.06	10.66 ± 1.30 ^{abc}	8.11 ± 2.36	6.67 ± 1.02 ^b
10	5.53 ± 0.96	8.96 ± 1.73 ^{abc}	7.64 ± 1.64	7.81 ± 1.53 ^b
13	8.05 ± 2.82	14.01 ± 3.24 ^a	10.71 ± 1.24	7.40 ± 1.67 ^b
16	5.70 ± 1.06	12.85 ± 3.02 ^{ab}	8.11 ± 2.11	5.74 ± 1.55 ^b
19	7.71 ± 2.84	8.04 ± 1.49 ^{abc}	8.06 ± 3.30	16.39 ± 3.65 ^a
21	6.97 ± 1.65	11.39 ± 2.32 ^{ab}	5.06 ± 1.89	7.39 ± 1.52 ^b

Values with different letter on the same column were statistically important ($P < 0.05$).

DISCUSSION

In this study, it was aimed to impregnate goats that had not been pregnant during the breeding season using an oestrus stimulation protocol to reveal the effect of GnRH injection at breeding and after breeding

on reproductive performance and embryonic loss. After synchronisation, various anti-luteolytic strategies can be used to support corpus luteum development and elevate progesterone concentration in the luteal phase to decrease embryonic loss and increase reproductive

performance [6]. Therefore, application of GnRH to support the luteal structure and increase progesterone levels may be beneficial in terms of supporting embryonic life [11].

Previous research has found that, in the anoestrus period, pregnancy rates of goats exposed to different durations of vaginal sponge for inducing oestrus vary in the range of 33-77% [1,5,19,29]. In this study, the pregnancy rates of the GnRH0, GnRH7, GnRH₀₊₇ and control groups were 33.3%, 38.5%, 25.9% and 46.2%, respectively ($P > 0.05$; Table 1). Akar *et al.* [2] reported that, after a short-term synchronisation protocol including intravaginal progesterone (for 7 days) and 500IU PMSG plus 75 µg of d-cloprostenol, the oestrus rate was 88.7%; furthermore, the pregnancy rate was 28.89% in the GnRH treatment group, in which GnRH injection was administered 12 days after breeding, whereas it was 37.21% in the control group with no GnRH treatment implemented. According to Sarıbay *et al.* [31], in the anoestrus period, after a long oestrus synchronisation protocol (14 days) involving progesterone, PMSG and cloprostenol, the oestrus rate was 100%; moreover, the pregnancy rate was 38.1% in the GnRH treatment group in which a single-dose GnRH injection was administered 48 h after withdrawal of the sponge, and in the control group, the pregnancy rate was 33.3%. Fonseca and Torres [18] reported that, following the oestrus synchronisation protocol involving intravaginal progesterone treatment for 9 days and 220IU PMSG plus d-cloprostenol, the oestrus rate was 83.1%, and the pregnancy rate of the hCG treatment group injected with hCG on day 5 after breeding was 50%; the pregnancy rate was 68% in the control group. In the present study, in goats having failure to conceive during the season in the breeding season, with the oestrus induction protocol including progesterone and 550IU PMSG plus 125 µg of d-cloprostenol, the oestrus rate was 96.23% in the treatment groups, whereas the pregnancy rate was 46.2% in the control group (Table 1). These results were observed to be similar to those of previous studies. In addition, the oestrus induction protocol used in goats with failure to conceive during the season can be considered successful.

Whereas oestrus induction was successful, GnRH application at different times after breeding did not improve the pregnancy rate. No statistical difference could be identified between the treatment groups in terms of fertility parameters (pregnancy

rate, multiple pregnancy rate, and litter size). GnRH agonists can affect the pituitary gland (PG) in different ways because of their dissimilar chemical structures. In addition, single GnRH injection can result in high gonadotropin release and an increased number of receptors (fivefold for follicle stimulating hormone [FSH] receptors and tenfold for luteinising hormone [LH] receptors). However, continuous treatment with GnRH agonists may also cause adverse effects by impairing LH release, leading to insensitisation of the PG to GnRH or decreasing the response of PG to GnRH [30]. In one study, higher levels of plasma progesterone concentration were detected in the treatment group (PMSG 500IU) compared with the control group [27]. It has also been reported that, because of the luteotrophic effect of PMSG used in oestrus induction protocols, the effects of GnRH and hCG can be masked by PMSG treatment [18]. In the present study, it was thought that the reason for the high fertility level in the control group may be due to the mentioned effects of GnRH.

In the out-of-season period, the onset of oestrus was 15.4-44.6 h after the withdrawal of IS. This variation resulted from different breeds, geographic locations and durations of IS treatment [12,17,23,25,33,34]. In this study, after withdrawal of the IS, the rate of oestrus onset at 36-48 h was 38.7% (41/106), that at 48-60 h was 21.7% (23/106), and that at 60 h and beyond was 35.8% (38/106). While the onset of oestrus in this study was similar to the results of some previous studies [17,33,34], in other research, the onset of oestrus was earlier than in the present findings [12,22,23,25]. It can be said that these differences may be caused by synchronisation procedures, hormone and breed differences. Alaçam *et al.* [3] stated that GnRH injection on day 12 post-breeding can improve fertility in Angora goats. Cam and Kuran [7] indicated that GnRH injections on day 12 post-breeding can improve breeding performance. In the same way, Uslu and Gülyüz [33] stated that GnRH injection after breeding has a positive effect on the pregnancy rate. In contrast, some studies have shown that hCG and GnRH injection after breeding are not beneficial for increasing fertility or the pregnancy rate [2,4,26,31]. This study does not support the hypothesis that GnRH treatment at breeding and after breeding prevents embryonic loss and improves reproductive performance. One study showed that the differences between previous research resulted

from different physiological statuses brought about by the different study designs, nutritional statuses, farm conditions and oestrus induction protocols in different works [16]. In addition, breed, time of treatment or dosage of drugs can have a critical effect on pregnancy rate and embryo viability. In the present study, the embryonic loss rates were 52.6%, 56.5%, 63.1% and 47.8% in the GnRH0, GnRH7, GnRH0+7 and control groups, respectively. No statistical difference could be found ($P > 0.05$; Table 1).

Some studies have reported that post-insemination GnRH treatment can be successful in preventing embryonic loss in sheep [8,16,21]. In this study, GnRH addition to the oestrus induction protocol was not effective in reducing embryonic death in goats. When the oestrus synchronisation protocol was implemented in the out-of-season period, embryonic loss was higher than that in the breeding period. Many processes, such as ovulation, fertilisation, corpus luteum lifespan and implantation, are the main reasons for embryonic loss underlain by. In the same way, the formation and lifespan of the corpus luteum in the breeding period have been found to be longer than that in the non-breeding period [28]. In one study, the plasma progesterone concentration after hCG treatment applied on the fifth day after breeding increased between day 7 (4.7 ± 1.9 and 3.9 ± 1.8 ng/mL) and day 13 (8.1 ± 3.2 and 5.2 ± 2.0 ng/mL) by up to 33% and 72% [18]. However, in the present study, progesterone concentration did not differ statistically on days 3, 6, 7, 10, 13, 16 and 19 ($P > 0.05$; Table 2).

When the groups were assessed in terms of progesterone concentration, no statistical difference was found in comparing the GnRH0 and GnRH₀₊₇ groups ($P > 0.05$; Table 2). However, the serum progesterone level was statistically different in the GnRH7 group compared with the control group ($P < 0.05$; Table 2).

In the GnRH₇ group, a peak level of progesterone was observed on day 13. This result was similar to that of a previous study [18].

CONCLUSION

Based on the present study's results, it was observed that GnRH treatment on day 7 post-breeding following oestrus induction including FGA and PMSG can increase serum progesterone levels in Damascus goats in the anoestrus period. However, following the oestrus induction in the anoestrus period, it was seen that GnRH treatment at breeding or on day 7 after breeding did not have any positive effect on embryonic loss and reproductive performance. The oestrus induction protocol can be implemented successfully, yielding a 35% pregnancy rate in Damascus goats in the anoestrus period, but embryonic loss must be deeply studied in detail.

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